Stem Cell Research 16 (2016) 342–344

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of KCL018 research grade human embryonic stem cell line carrying a mutation in the *DMPK* gene

Cristian Miere, Heema Hewitson, Liani Devito, Victoria Wood, Neli Kadeva, Glenda Cornwell, Stefano Codognotto, Emma Stephenson, Dusko Ilic *

Stem Cell Laboratories, Division of Women's Health, Faculty of Life Sciences and Medicine, King's College London and Assisted Conception Unit, Guys' Hospital, London, United Kingdom

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 5 January 2016
Accepted 12 January 2016
Available online 15 January 2016

ABSTRACT

The KCL018 human embryonic stem cell line was derived from an embryo donated for research that carried an autosomal dominant mutation affecting one allele of the *DMPK* gene encoding the dystrophia myotonica protein kinase (2200 trinucleotide repeats; 14 for the normal allele). The ICM was isolated using laser microsurgery and plated on γ-irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment. Pluripotent state and differentiation potential were confirmed by in vitro assays.

Resource table

Name of stem cell line	KCL018
Institution	King's College London, London UK
Derivation team	Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson
Contact person and email	Dusko Ilic, email: dusko.ilic@kcl.ac.uk
Type of resource	Biological reagent: cell line
Sub-type	Human pluripotent stem cell line
Origin	Human embryo
Key marker expression	Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity
Authentication	Identity and purity of line confirmed
	1) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy. 14 (1), 122–128.
Link to related literature (direct URL links and full references)	doi: 10.3109/14,653,249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654
	2) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381. doi: 10.1038/nprot.2012.080 http://www.ncbi.nlm.nih.gov/pubmed/22722371
Information in public databases	KCL018 is a National Institutes of Health (NIH) registered hESC line NIH Registration Number: 0218 NIH Approval Number: NIHhESC-13-0218 http://grants.nih.gov/stem_cells/registry/current.htm?id=658
Ethics	The hESC line KCL018 is derived under license from the UK Human Fertilisation and Embryology Authority (research license numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90). Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation.

Resource details

Consent signed	Aug 12, 2009
Embryo thawed	Aug 23, 2009
UK Stem Cell Bank Deposit Approval	Sep 23, 2010 Reference: SCSC10-30
Sex	Female 46, XX
Grade	Research
Disease status (Fig. 1)	Mutation affecting one allele of the *DMPK* gene encoding dystrophia myotonica protein kinase (~2200 CTG repeats; 14 for the normal allele) associated with Myotonic dystrophy Type 1 (Ilic et al., 2012)
Karyotype (aCGH)	No copy number changes detected
DNA fingerprint	Allele sizes (in bp) of 17 microsatellite markers

* Corresponding author.
E-mail address: dusko.ilic@kcl.ac.uk (D. Ilic).

http://dx.doi.org/10.1016/j.scr.2016.01.004

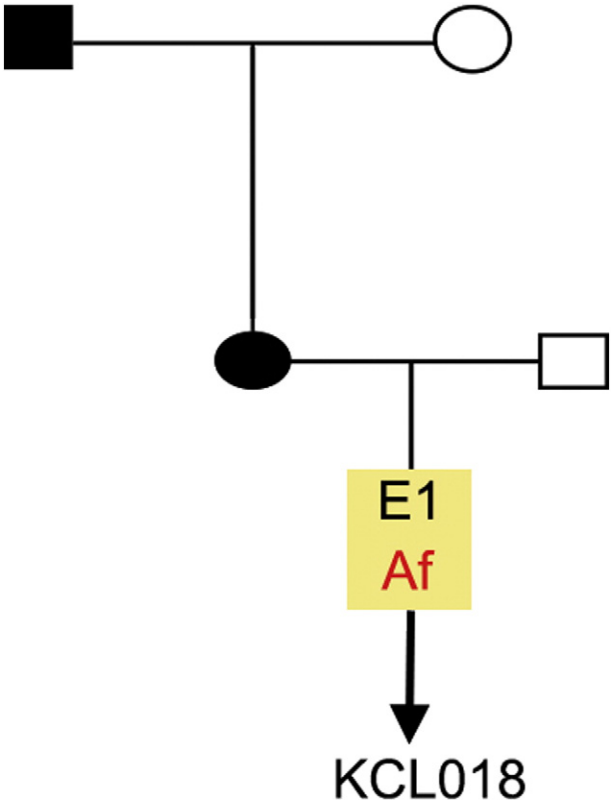

Fig. 1. Genetic pedigree tree. The couple undergoing IVF had only on embryo in this particular cycle. The embryo carried the mutation and was donated for research.

	specific for chromosomes 13, 18 and 21 (Ilic et al., 2012)
Viability testing	Pass
Pluripotent markers (immunostaining) (Fig. 2)	NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity (Ilic et al., 2012)
Three germ layers	Endoderm: AFP (α-fetoprotein); Ectoderm: TUBB3 (tubulin, β3 class III); Mesoderm: ACTA2 (actin, α2, smooth muscle) (Ilic et al., 2012)
differentiation in vitro (immunostaining) (Fig. 3)	
Sibling lines available	No

We generated KCL018 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012). The expression of the pluripotency markers was tested after freeze/thaw cycle (Ilic et al., 2012). Differentiation potential into three germ layers was verified in vitro (Ilic et al., 2012).

Materials and methods

Consenting process

We distribute Patient Information Sheet (PIS) and consent form to the in vitro fertilization (IVF) patients if they opted to donate to research embryos that were stored for 5 or 10 years. They mail signed consent back to us and that might be months after the PIS and consent were mailed to them. If in the meantime new versions of PIS/consent are implemented, we do not send these to the patients or ask them to resign; the whole process is done with the version that was given them initially. The PIS/consent documents (PGD-V.6) were created on Aug. 10, 2007. HFEA Code of Practice that was in effect at the time of document creation: Edition 7 — R.1 (http://www.hfea.gov.uk/2999.html). The donor couple signed the consent on Oct. 15, 2009. HFEA Code of Practice that was in effect at the time of donor signature: Edition 8 — R.1. HFEA Code of Practice Edition 7 — R.1 was in effect until Dec. 09, 2007 and Edition 8 — R.1 was in effect: Oct. 01, 2009–Apr. 06, 2010.

Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in details (Ilic et al., 2012; Stephenson et al., 2012). The cellular area containing the ICM was then washed and transferred to plates containing mitotically inactivated human neonatal foreskin fibroblasts (HFF).

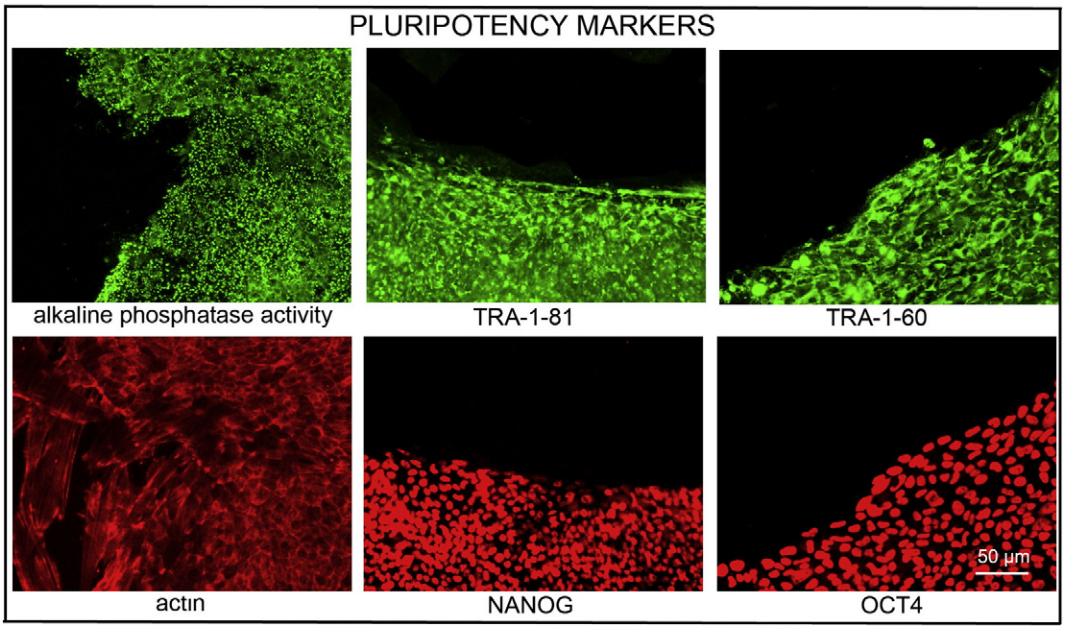

Fig. 2. Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (Oct4, Nanog, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Actin stress fibers, visualized with rhodamine-phalloidin (red), are present in both feeders and hES cell colonies, whereas AP activity (green) is detected only in hES cells. Scale bar, 50 µm.

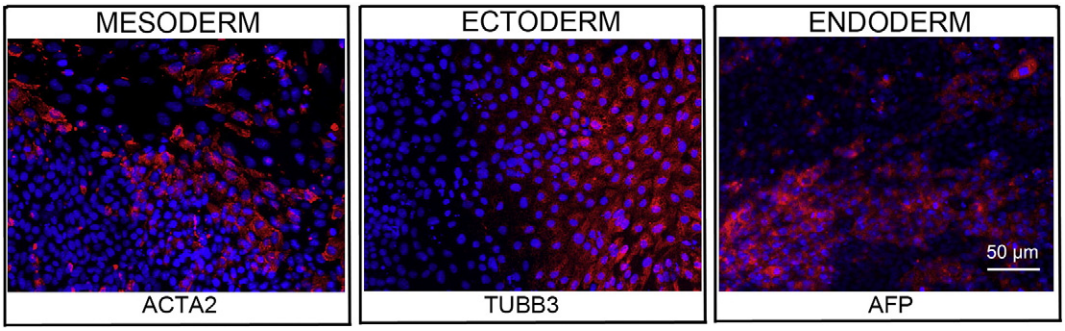

Fig. 3. Differentiation of three germ layers in vitro is confirmed by detection of markers: smooth muscle actin (red) for mesoderm, β-III tubulin (red) for ectoderm and α-fetoprotein (red) for endoderm. Nuclei are visualized with Hoechst 33,342 (blue). Scale bar, 50 µm.

Cell culture

ICM plated on mitotically inactivated HFF were cultured as described (Ilic et al., 2012; Stephenson et al., 2012). TE cells were removed mechanically from outgrowth (Ilic et al., 2007; Ilic et al., 2010). hESC colonies were expanded and cryopreserved at the third passage.

Viability test

Straws with the earliest frozen passage (p. 2–3) are thawed and new colonies are counted three days later. These colonies are then expanded up to passage 8, at which point cells were part frozen and part subjected to standard battery of tests (pluripotency markers, in vitro and in vivo differentiation capability, genetics, sterility, mycoplasma).

Pluripotency markers

Pluripotency was assessed using two different techniques: enzymatic activity assay [alkaline phosphatase (AP) assay] and immunostaining as described (Ilic et al., 2012; Stephenson et al., 2012).

Genotyping

DNA was extracted from hES cell cultures using a Chemagen DNA extraction robot according to the manufacturer's instructions. Amplification of polymorphic microsatellite markers was carried out as described (Ilic et al., 2012). Allele sizes were recorded to give a unique fingerprint of each cell line.

Array comparative genomic hybridization (aCGH)

aCGH was performed as described in details (Ilic et al., 2012).

Chr	Marker	Allele 1	Allele 2
	D13S252	299	299
	D13S305	443	458
13	D13S325	289	297
	D13S628	429	450
	D13S634	397	417
	D18S386	383	386
	D18S390	372	372
	D18S391	209	217
18	D18S535	482	486
	D18S819	408	424
	D18S976	479	479
	D18S978	219	219
	D21S11	244	251
	D21S1409	216	224
21	D21S1411	308	308
	D21S1435	184	188
	D21S1437	331	335

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

This work was supported by the UK Medical Research Council grants G0701172 and G0801061. We thank Dr. Yacoub Khalaf, Director of the Assisted Conception Unit of Guy's and St Thomas' NHS Foundation Trust and his staff for supporting the research program. We are especially indebted to Prof Peter Braude and to the patients who donated embryos.

References

Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy 14 (1), 122–128.

Ilic, D., Caceres, E., Lu, S., Julian, P., Foulk, R., Krtolica, A., 2010. Effect of karyotype on successful human embryonic stem cell derivation. Stem Cells Dev. 19 (1), 39–46.

Ilic, D., Genbacev, O., Krtolica, A., 2007. Derivation of hESC from intact blastocysts. Curr. Protoc. Stem Cell Biol. (Chapter 1: Unit 1 A.2).

Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381.